Differential Neurochemical Responses of the Canine Striatum with Pentobarbital or Ketamine Anesthesia: A 3T Proton MRS Study

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ABSTRACT. Although anesthetic agents are known to affect cerebral metabolism, pentobarbital and ketamine have been widely used for animal imaging studies. The purpose of this study is to evaluate alterations in striatum metabolites in dogs between anesthetized with pentobarbital and with ketamine in proton magnetic resonance spectroscopy (^{1}H-MRS). ^{1}H-MRS was performed to ten healthy adult beagle dogs (9–11 kg) at a field strength of 3 T in order to identify metabolic changes after pentobarbital or ketamine administration in the striatum *in vivo*. Ten dogs were divided into 2 groups as follows: 5 as the pentobarbital-administered group (P group) and 5 as the ketamine-administered group (K group). We found that levels of Glx of the P group was significantly lower than that of the K group (6.90 ± 0.99 (SD) vs 9.77 ± 1.14 in 5 dogs, p= 0.003). In addition, the P group also has lower levels of Cr (6.29 ± 0.44 vs 7.89 ± 0.91 in 5 dogs, *p*=0.009) and NAA (5.02 ± 0.65 vs 6.45 ± 1.13 in 5 dogs, *p*=0.041) compared to the K group. However, there were no significant difference between the P group and the K group in striatal levels of Cho and Ins (*p*>0.1). We demonstrated that MRS-measured metabolites in the specific regions of the brain can be influenced by anesthetic agents.
KEY WORDS: canine, ketamine, MR spectroscopy, pentobarbital, striatum.

Studies in neuroscience and anesthesiology have focused on the modulation of synaptic communication that involves neurotransmitters induced by anesthetic drugs [12]. These anesthetics modify the release and concentration of neurotransmitters at specific regions in the brain [1].

Positron emission tomography (PET), single-photon emission-computed tomography (SPECT), and proton magnetic resonance imaging ([1]H-MRI) and spectroscopy (^{1}H-MRS) offer noninvasive methods for functional and metabolic/biochemical evaluation of the brain [17]. One of these methods, *in vivo* single-voxel ^{1}H-MRS, is now a widely available method on MRI scanners used for experimental and clinical studies [24]. ^{1}H-MRS can measure the levels of several brain metabolites, including creatine (Cr)/phosphocreatine (PCr), choline-containing compound (Cho), and N-acetyl-aspartate (NAA) [24]. In this respect, MRS can offer a noninvasive and efficient means for performing functional and metabolic/biochemical evaluation of the brain, which may be useful in the diagnosis and follow-up of metabolic and degenerative diseases [4].

The striatum is the major input station of the basal ganglia system. It is involved in Parkinson's disease, Huntington's disease, choreas, choreoathetosis and dyskinesias [17]. Recently, ^{1}H-MRS studies of common and severe neuropsychiatric disorders (e.g., obsessive-compulsive disorder, schizophrenia, etc.) have reported abnormal metabolite levels in the striatum (cudate and putamen nuclei) [24]. Therefore, we considered that the evaluation of the striatum with ^{1}H-MRS may be an important portion of neuroscience, and we decided to investigate the changes of striatal metabolites in this study.

Because imaging of small animals generally requires anesthesia, anesthetic agents can induce unintended effects on animal physiology that may confound the results of imaging studies [8]. The use of pentobarbital and ketamine anesthesia is popular in imaging studies of laboratory animals. Pentobarbital is a short-acting barbiturate that both enhances and mimics the action of the neurotransmitter gamma-aminobutyric acid (GABA), the principal inhibitory neurotransmitter in the central nervous system (CNS) [15]. However, ketamine appears to exert the majority of its CNS actions via its antagonistic effect at N-methyl-D-aspartate (NMDA) receptors [16]. Other evidence suggests that it may have effects at other receptors, such as the glutamate receptor [14]. Ketamine has been termed a "dissociative" anesthetic because patients who receive ketamine alone appear to be in a cataleptic state. The effects of ketamine on the gross electrical activity of the brain are markedly different from those of "depressant" anesthetics, such as pentobarbital [21]. We hypothesized that the differences in anesthetic mechanisms between dissociative and depressant anesthetics are due to different effects on the brain as seen

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on ^{1}H-MRS of the striatum. Therefore, we expect to determine whether the administration of pentobarbital or ketamine has any effect on striatum spectral metabolites in dogs. The selection of voxel-of-interst (VOI) around the striatum with the human 3T MR system has limitation in animals brain size. Thus the dog was chosen due to its advantage of brain size for rather than rodents or other small laboratory animals.

The purpose of this study was to evaluate alterations in striatum metabolites of dogs between anesthetized with pentobarbital and with ketamine in proton magnetic resonance spectroscopy (^{1}H-MRS), and to investigate the appropriateness of anesthetic agents for ^{1}H-MRS study.

MATERIALS AND METHODS

Experimental design: Ten healthy beagle dogs (18 ± 6 months, weight 9.7 ± 0.9 kg) were used without sex discrimination. All dogs were considered to be normal following physical, hemodiagnostic (complete blood count), and blood chemistry examination. Each animal was individually housed according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, under an approved protocol from the Institutional Laboratory Animal Care and Use Committee of Konkuk University. Ten dogs were divided into two groups: 1) P group, N=5; 2) K group, N=5. All animals were premedicated with intramuscular medetomidine (40 μg/kg, DOMITOR®, Pfizer Korea, Ltd.) to maintain anesthesia for 1 hr and to induce skeletal muscle relaxation. After 15 min, 5 beagles were anesthetized with intravenous pentobarbital sodium (8 mg/kg, ENTOBAR®, Hanlim PHARMA Co., Ltd.) (P group). The 5 other beagles were anesthetized with intravenous ketamine HCl (15 mg/kg, KETAMIN 50®, Yuhan Co., Ltd.) (K group). During MR experiments, all animals were taken to stage III plane 3 of anesthesia. It was characterized by decreased intercostal muscle function and tidal volume, increased respiration rate, profound muscle relaxation, diaphragmatic breathing, a weak corneal reflex, and a centered and dilated pupil. There was no need to give additional administration.

Localized ^{1}H-MRS: ^{1}H-MRS measurements were performed in the P group and K group. MR experiments were conducted using a Philips Achieva 3 Tesla System (Philips Healthcare, Best, The Netherlands) with an 8 channel SENSE knee coil. Anesthetized animals were placed in a sternal position. A scout image was initially obtained to verify the position of the subject and the image quality. The position of the volume-of-interest (VOI) was carefully selected based on multislice transverse, coronal, and sagittal T2-weighted MR images obtained using a turbo spin echo (TSE) sequence (TR=4540 ms, TE=80 ms, slice thickness=2.0 mm, NEX=2, matrix size=144 × 128, reconstruction matrix 240 × 240, SENSE factor=2); a rectangular VOI (10 × 20 × 10 mm^3) was placed in the striatum. As a single voxel technique, ^{1}H-MRS spectra were obtained through a STEAM sequence performed according to the following

parameters: data point 1024, spectra BW=2,000 Hz, TR=2,000 ms, TE=30 ms, NSA=128. The relative metabolite levels for N-acetylaspartate (NAA), glutamine and glutamate complex (Glx), creatine (Cr), choline-containing compounds (Cho), and myoinositol (Ins) were determined.

^{1}H-MRS Data Analysis: *In vivo* proton spectra were analyzed using LCModel [19, 20], which calculates the best fit to the experimental spectrum as a linear combination of model spectra (simulated spectra of brain metabolites). Raw data (FIDs) were used as standard data input. The water-suppressed time domain data were analyzed between 0.2 ppm and 4.0 ppm without further T_1 and T_2 correction. The following 17 metabolites were included in the basis set: alanine (Ala), aspartate (Asp), creatine (Cr), γ-aminobutyric acid (GABA), glucose (Glc), glutamate (Glu), glutamine (Gln), glutathione (GSH), glycerophosphorylcholine (GPC), phosphorylcholine (PCho), myo-inositol (mIns), lactate (Lac), N-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG), phosphocreatine (PCr), scyllo-inositol (Scy) and taurine (Tau). In addition, macromolecule was also included in the basis set. The *in vivo* proton spectra were accepted if the signal-to-noise ratio (SNR) was 6 or more, and the standard deviation of the fit for the metabolite was < 20% (Table 1).

Statistical analysis: Data are presented as means ± SDs. Statistical analysis was performed with commercial SPSS software (SPSS 15.0 for Windows, SPSS, Chicago, IL U.S.A.). The two-tailed *t*-test with independent factors was used to evaluate differences between MRS data. *p*-values < 0.05 were considered statistically significant.

RESULTS

Figure 1 shows the ^{1}H-MRS fitted by the LCModel (Version 6.2–1L) quantification algorithm. Well-defined peaks were detected for all normal metabolic products measured, indicating acceptable signal-to-noise ratios generated by the number of acquisitions used for the selected VOI. Figure 2 shows the levels of Cr, NAA, Glx, Cho, Ins between the P group and K group. We found that levels of Glx of the P group were significantly lower than that of the K group (6.90 ± 0.99 (SD) vs 9.77 ± 1.14 in 5 dogs, p=0.003). In addition, the P group also has lower levels of

Table 1. The levels of each striatal metabolites in canine brain with ^{1}H-MRS

	Concentration		CV[a] (%)	P-value
	Pentobarbital	Ketamine		
Cr	6.29 ± 0.44[b]	7.85 ± 0.91	6.3	0.009*
NAA	5.02 ± 0.65	6.45 ± 1.13	8.7	0.041*
Glx	6.9 ± 0.99	9.77 ± 1.14	17.8	0.003*
Cho	2.28 ± 0.24	2.58 ± 0.36	6.1	0.157
Ins	4.26 ± 0.65	4.18 ± 0.88	14.9	0.879

* P<0.05, applying the two-tailed t-test with independent factors.
a) Cofficients of variations (standard deviation over mean).
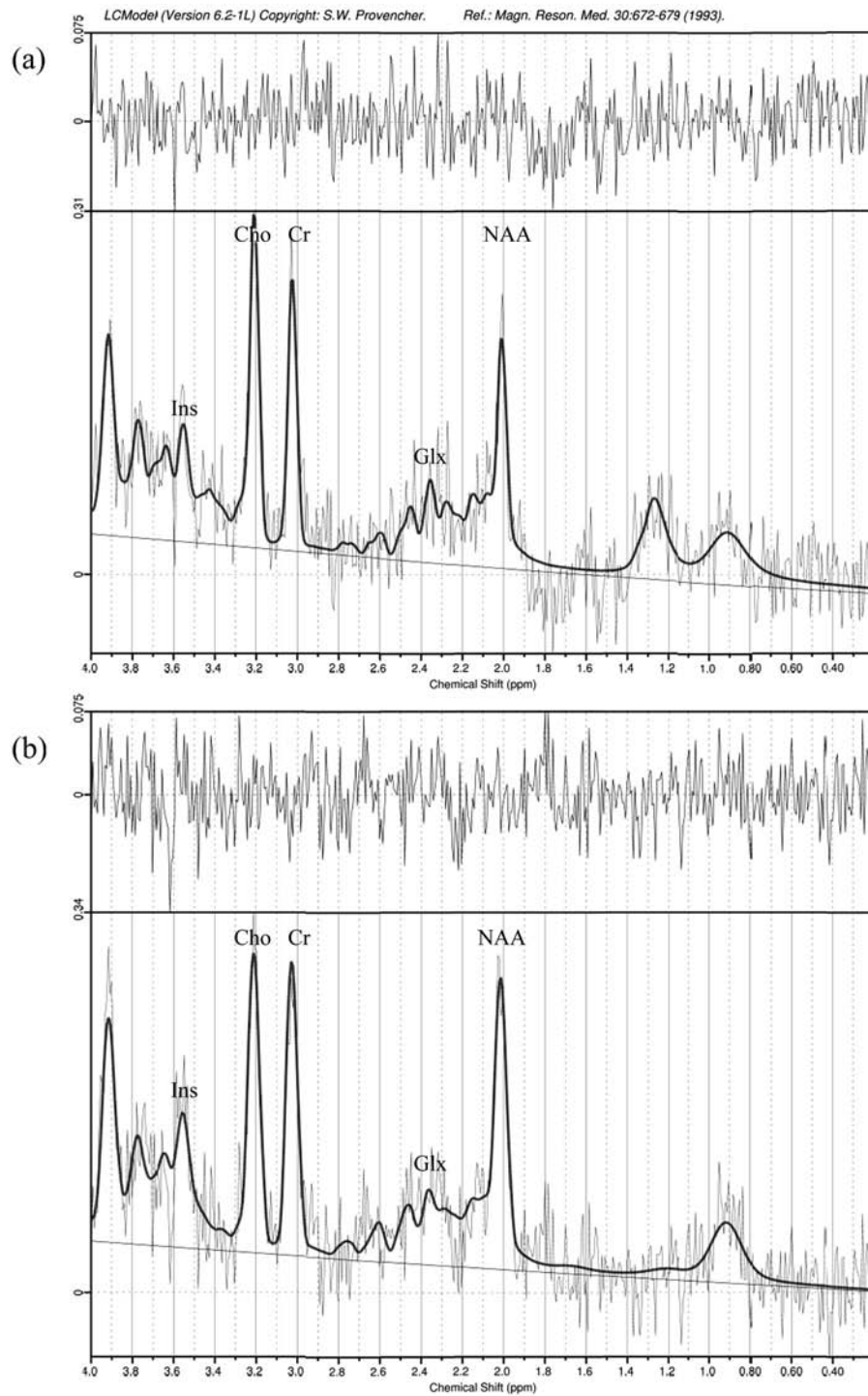b) Arithmetic mean ± standard deviation.

Fig. 1. Typical analysed *in vivo* striatum ¹H-MRS spectrum of the canine brain. On the bottom panel the acquired spectrum is shown (without line bradening, gence the more noisy appearance) super-imposed with the bold fitted spectrum from LCModel (Version 6.2-1L). Residuals (observed-fitted) are presented in the top panel. (a) The spectra from the canine anesthetized with pentobarbital. (b) The spectra from the canine anesthetized with ketamine.
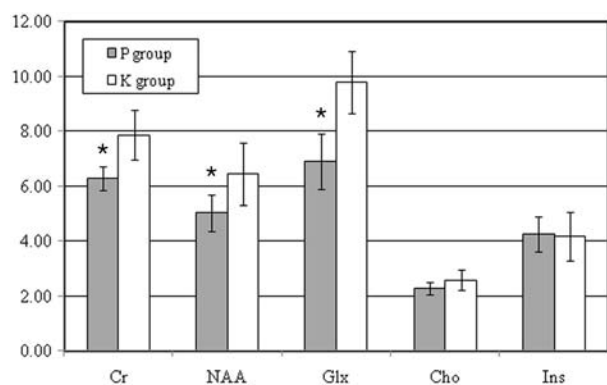
Fig. 2. Comparison of the relative ratios between the P group and the K group. Each of the gray and white bars represents the P group and K group, respectively. *p*-values less than 0.05 were considered statistically significant. This graph shows that the levels of Glx, Cr, and NAA were significantly lower in the P group than it was in the K group (*p*=0.003, *p*=0.009, *p*=0.041). However, there were no significant difference between the P group and the K group in the striatal levels of Cho and Ins (*p*>0.1).

Cr (6.29 ± 0.44 vs 7.89 ± 0.91 in 5 dogs, *p*=0.009) and NAA (5.02 ± 0.65 vs 6.45 ± 1.13 in 5 dogs, *p*=0.041) compared to the K group. However, there were no significant difference between the P group and the K group in striatal levels of Cho and Ins (*p*>0.1) .

DISCUSSION

Pentobarbital and ketamine were usually administered for anesthesia with experimental ^{1}H-MRS study. However, there has been no report concerning the effects of these anesthetics in the striatum in ^{1}H-MRS studies.

In this study, we found that anesthetic agents significantly influenced the levels of Glx (*p*=0.003), Cr (*p*=0.009) and NAA (*p*=0.041) in the striatum. The Glx complex consists of glutamine (Gln) and glutamate (Glu). Gln is the cellular energy source [18] and Glu is the primary excitatory neurotransmitter [23]. The glucose converts to Glu by its brain metabolism [7]. Pentobarbital has the effect of Glu receptor blockade [9], and reduces cerebral metabolic rate of glucose [18]. We thinks that pentobarbital reduces the striatal Glx levels in this manner. Ketamine, on the other hand, evokes a significant release of Glu in prefrontal cortex [11], increases concentrations of extracellular dopamine (DA) in the striatum and prefrontal cortex [23], decreases DA receptor availability in the striatum [23], and reduces levels of the vesicular glutamate transporter in striatum [10]. Glu release from corticostriatal fibers is dependent on the striatal DA and may be regulated by D2 receptors located on the corticostriatal terminals [3]. Therefore, our results reveal that the administration of ketamine induces to increase striatal Glx levels.

In practice, cerebral concentrations of total Cr remain relatively constant, while changes in NAA, either as an absolute concentration or as a ratio between NAA/Cr, are diagnostically important [13]. Although this is a standard method to estimate changes in metabolites, it has some restrictions. Some studies have shown that the Cr level is not as stable as previously thought. Therefore, using only the NAA/Cr ratio might lead to false results, as Cr may be an inconsistent denominator [22]. In this study, the striatal levels of Cr were changed by administration of two different anesthetic agents. So we demonstrate that the levels of Cr are not stable in anesthetic conditions.

Pentobarbital administration decreases the extracellular concentration of DA in the striatum in a dose-dependent manner [2]. Although, ketamine has been shown to increase extracellular concentration of DA [5]. Ellis *et al.* reported that lower dopaminergic activity in the striatum may be associated with reduced NAA/Cho ratios [6]. The exact physiological mechanism of this possible association between dopaminergic activity and the NAA level remains unresolved and merits further investigation [22].

This study revealed that MRS-measured metabolites in specific regions of the brain can be influenced by anesthetic agents. Therefore, It is necessary to consider the careful choice of anesthetic agent in ^{1}H-MRS studies. We acknowledge several limitations in our study. First, the number of subjects was small. Data must be acquired in more subjects to substantiate the preliminary findings of alterations with pentobarbital or ketamine administration, and further, must examine the relationship between dose dependant effects of each drugs on ^{1}H-MRS. Second, we had premedicated the animal with alpha-2 adrenergic agonist (medetomidine) because satisfactory anesthesia could not be maintained with pentobarbital or ketamine administration alone during the ^{1}H-MRS study. In this reason, the *ex vivo* NMR study to measure the normal range of metabolites without any anesthetic agents or the effect of single anesthetic agents requires future investigation.

CONCLUSION

In summary, imaging of small animals generally requires anesthesia. Sedation is also frequently used for ^{1}H-MRS in children. We have demonstrated that MRS-measured metabolites in specific regions of the brain can be differentially influenced by anesthetic agents. This study showed that the choice of anesthetic is significant in the setting of ^{1}H-MRS. Appropriate anesthetic choice should be pursued in order to exclude the effect of anesthetic agents on the target area.

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